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TITLE: LIMITED TOXICITY AND MUTAGENICITY TESTING OF FIVE UNICHARGE PROPELLANT COMPOUNDS

SUBTITLE: Evaluation of Two Unicharge Propellants in the Primary Eye Irritation

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CONTRACTING ORGANIZATION: Pharmakon Research International, Inc.
P.O. Box 609
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13. ABSTRACT *(Maximum 200 words)*

Bis (2,2-dinitropropyl) acetal/formal (~50/50 mixture) ± diphenyl amine stabilizer (BDNPA/F±DPA) were tested for eye irritation. One group of six rabbits per study were administered 0.1 mL of the test article directly into the right eye. The treated eyes were examined at 1, 24, 48 and 72 hours after treatment and grades of ocular reaction were recorded. Positive ocular scores were observed in both treatment groups at the 1 hour observation period. All scores were normal in both treatment groups for the remainder of the study. Based upon these observations BDNPA/F±DPA were determined to be eye irritants. The Toxicity Category for Eye Irritation for both test articles is Class IV.

14. SUBJECT TERMS

Eye Irritation, Unicharge, Propellants, bis(2,2-dinitropropyl) acetal, bis-(2,2-dinitropropyl) formal, lab animal, RA III

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FOREWORD

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____ For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

____ In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

____ In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

____ In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

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Evaluation of Two Unicharge Propellants in the Primary Eye Irritation

EXECUTIVE SUMMARY

Test articles bis-(2,2-dinitropropyl) acetal with diphenyl amine stabilizer and bis-(2,2-dinitropropyl) formal without diphenyl amine stablizer were instilled in the right eye of six rabbits each at 0.1 mL/treated eye. The eyes were examined at 1, 24, 48 and 72 hours after administration.

Postive ocular scores of the conjunctivae were observed in four bis-(2,2-dinitropropyl) acetal with diphenyl amine stabilizer treated animals at the 1 hour observation period. The remaining two animals exhibited vessels injected above normal at the 1 hour observation period. All scores returned to normal at 24 hours.

Positive ocular scores of the conjunctivae were observed in two bis-(2,2-dinitropropyl) formal without diphenyl amine stablizer treated animals at the 1 hour observation period. The remaining four animals exhibited vessels injected above normal at the 1 hour observation period. All scores returned to normal at 24 hours. Both treatment groups were terminated following the 72 hour observation period.

Based upon the observations made in the Primary Eye Irritation studies in rabbits, bis-(2,2-dinitropropyl) acetal with diphenyl amine stabilizer and bis-(2,2-dinitropropyl) formal without diphenyl amine stablizer were determined to be eye irritants. The Toxicity Category for Eye Irritation for both test articles is Class IV (minimal effects cleaning in less than 72 hours).

Evaluation of Two Unicharge Propellants
in the Primary Eye Irritation

PH 421-US-001, 002-91

STUDY DESCRIPTION

Sponsor:	U.S. Army Medical Research and Development Laboratory Fort Detrick Frederick, MD 21702-5010
Testing Facility:	Pharmakon Research International, Inc. P.O. Box 609 Waverly, PA 18471
Test Facility Study Conduct S.O.P. No.:	PH-421
Study Numbers:	PH 421-US-001-91 PH 421-US-002-91
Purpose of the Study:	To determine the irritant and/or corrosive effects on eyes of rabbits.
Ownership of the Study:	The sponsor owns the study. All raw data, analyses and reports are the property of the sponsor.
Study Monitor:	Major Nathaniel Powell, U.S. Army Medical Research and Development Laboratory
Study Director:	Victor T. Mallory, B.S., RLAT, Pharmakon Research International, Inc.
Technical Performance:	Thomas O'Neill, B.S., LAT and Kim DiLeo, B.S., LAT
Q.A.U. Responsible Personnel:	Leslie J. Pinnell, M.S.
Date Study Director Signed Protocols:	September 23, 1991
Dates of Technical Performance:	**PH 421-US-001-91** - November 22, 1991 through November 25, 1991 **PH 421-US-002-91** - November 22, 1991 through November 25, 1991

Evaluation of Two Unicharge Propellants
in the Primary Eye Irritation
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Good Laboratory Practices Statement: These studies were conducted in compliance with the Good Laboratory Practice Regulations. There were no deviations from the GLP Regulations which affected the quality or integrity of the study. Q.A.U. findings from the inspections conducted of this study and from the audit of the final report are documented and have been provided to the study director and the test facility management.

Records Maintained: All raw data, final report documentation and protocol will be maintained in the archives of Pharmakon Research International, Inc.

Recordings: Standard Pharmakon Notebook

Notebook Reference: Notebook #1449, pages 173-174, 176-177

TEST ARTICLES

TEST ARTICLE	DESCRIPTION	LOT #	pH	CAS #	DATE SUBMITTED
bis-(2,2-dinitropropyl) acetal with diphenyl amine stabilizer (BDNPA/F+DPA)	yellow liquid	Set #1	5	5108-69-0	9/19/91
bis-(2,2-dinitropropyl) formal without diphenyl amine stabilizer (BDNPA/F-DPA)	yellow liquid	Set #2	5	5917-61-3	9/19/91

Analysis of Purity: The purity, identity, strength and stability of the test articles were the responsibility of the sponsor.

Stability: There was no apparent change in the physical appearance of the test articles during administration.

TEST SYSTEM

Species: Rabbit

Strain: New Zealand White

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Supplier (Source):	CAMM Research Lab Animals, Wayne, NJ
Sex:	Male and female
Age at Initiation:	8-12 weeks
Weight Range:	1.741-1.992 kilograms
No. on Study:	Six (6) (three males and three females) per study
Method and Justification for Randomization:	Selection of rabbits based upon body weight.
Acclimation Period:	Minimum of five (5) days
System of Identification:	Cage cards were marked with the study number, animal number, dose level and sex. Rabbits were ear tagged.

HUSBANDRY

Research Facility Registration:	U.S.D.A. Registration No. 23-R-107 under the Animal Welfare Act 74: SC 2131 et seq.
Animal Rooms:	Separate isolation by test system Light cycle - 12 hours light, 12 hours dark Temperature/Relative Humidity - Every attempt was made to maintain a temperature of 20°C ± 3°C (63-73°F) and a relative humidity of 30 to 70%. Any excursions outside the temperature or humidity ranges were of small magnitude and/or brief duration and did not adversely affect the validity of the study.
Housing:	Rabbits were housed individually in cages sized in accordance with the "Guide for the Care and Use of Laboratory Animals" of the Institute of Laboratory Animal Resources, National Research Council.
Sanitization:	Waste material was removed twice weekly. Cages and feeders were sanitized every two weeks.
Food:	Purina Lab Rabbit Chow H.F.® ad libitum. Food was checked daily and added or replaced as

needed. Feeders are designed to reduce soiling, bridging and scattering.

Food Analysis: There were no contaminants that were reasonably expected to be present in the dietary material known to be capable of interfering with the purpose or conduct of the study.

Water: Fresh tap water, ad libitum.

Water Analysis: Water is monitored for contaminants at periodic intervals according to Standard Operating Procedure PH-018.

METHODS

Rationale for Test System: A variety of experimental animals have been used, but it is recommended that testing will be performed using healthy adult albino rabbits. Commonly used laboratory strains will be used.

Compound Preparation: The test articles were dosed as received.

Dose Administration: 0.1 mL/treated eye

Rationale for Dose Selection: According to the EPA Federal Register, Vol. 50, No. 188, Friday, September 27, 1985.

Route of Administration: The test articles were administered directly into the eye.

Rationale for Route of Administration: To evaluate the irritant potential of the test article on the eye.

Frequency of Administration: Once (1) per test article

No. of Animals Per Dose Group: Six (6)

No. & Code of Dose Groups:

Rabbit No.	Dose
5501-5506	0.1 mL/treated eye [bis-(2,2-dinitropropyl) acetal with diphenyl amine stabilizer]
5291-5296	0.1 mL/treated eye [bis-(2,2-dinitropropyl) formal without diphenyl amine stablizer]

Evaluation of Two Unicharge Propellants
in the Primary Eye Irritation
PH 421-US-001, 002-91

Length of Studies: Seventy two (72) hours

Method of Study Performance: Both eyes of each experimental animal provisionally selected for testing were examined within 24 hours before testing started by the same procedure used during the test examination. Animals showing eye irritation, ocular defects or pre-existing corneal injury were not used. The test substance was placed in the conjunctival sac of the right eye of each animal after gently pulling the lower lid away from the eyeball. The lids were then gently held together for about one second in order to limit loss of the material. The other eye, which remained untreated, served as a control.

Type and Frequency of Test, Analysis and Measurements to be Made: The eyes were examined at 1, 24, 48 and 72 hours after treatment. The grades of ocular reaction were recorded at each examination period.

Data Analysis: Scoring and grading of irritation is according to the method of Draize, J.H. (1965), Appraisal of the Safety of Chemicals in Food, Drugs and Cosmetics - Dermal Toxicity, pages 49-52. Association of Food and Drug Officials of the U.S., Topeka, Kansas. Classification of Toxicity Categories is according to Addendum 2 on Pesticides Assessment Guidelines - Eye Irritation (U.S.) Environmental Protection Agency Washington, D.C., January 1988.

RESULTS

Positive ocular scores of the conjunctival were observed in four bis-(2,2-dinitropropyl) acetal with diphenyl amine stabilizer treated animals at the 1 hour observation period. The remaining two animals exhibited vessels injected above normal at the 1 hour observations period. All scores returned to normal at 24 hours.

Positive ocular scores of the conjunctivae were observed in two bis-(2,2-dinitropropyl) formal without diphenyl amine stablizer treated animals at the 1 hour observation period. The remaining four animals exhibited vessels injected above normal at the 1 hour observation period. All scores returned to normal at 24

hours. Both treatment groups were terminated following the 72 hour observation.

CONCLUSIONS

Based upon the observations made in the Primary Eye Irritation, bis-(2,2-dinitropropyl) acetal with diphenyl amine stabilizer and bis-(2,2-dinitropropyl) formal without diphenyl amine stablizer were determined to be eye irritants. The Toxicity Category for Eye Irritation for both test articles is Class IV (minimal effects cleaning in less than 72 hours).

Evaluation of Two Unicharge Propellants
in the Primary Eye Irritation
PH 421-US-001, 002-91

TABLE I

Scale for Scoring Ocular Lesions*

(1) Cornea
  (A) Opacity-degree of density (area most dense taken for reading)
    No opacity.....0
    Scattered or diffuse area, details of iris clearly visible.....1**
    Easily discernible translucent areas, details of iris slightly obscured..... 2
    Opalescent areas, no details of iris visible, size of pupil barely discernible.....3
    Opaque, iris invisible.....4

(2) Iris
  (A) Values
    Normal.....0
    Folds above normal, congestion, swelling, circumcorneal injection (any or all of these or combination of any thereof) iris still reacting to light (sluggish reactions are positive).....1**
    No reaction to light, hemorrhage, gross destruction (any or all of these).....2

(3) Conjunctivae
  (A) Redness (refers to palpebral and bulbar conjunctivae excluding cornea and iris)
    Vessels normal.....0
    Vessels definitely injected above normal.....1
    More diffuse, deeper crimson red, individual vessels not easily discernible.....2**
    Diffuse beefy red.....3
  (B) Chemosis
    No swelling.....0
    Any swelling above normal (includes nictitating membrane.....1
    Obvious swelling with partial eversion of lids.....2**
    Swelling with lids about half closed.....3
    Swelling with lids about half closed to completely closed.....4

* Draize, J.H., et al., J. Pharm, Exp. Ther. 82:377-390, 1944.
**Figures indicates lowest grades considered positive under the Federal Hazardous Substances Act Regulations at 16 CFR 1500.42

TABLE I (continued)

Toxicity Categories for Eye Irritation

I	II	III	IV
Corrosive (irreversible destruction of ocular tissue) or corneal involvement or irritation persisting for more than 21 days	Corneal involvement or irritation clearing in 8-21 days	Corneal involvement or irritation clearing in 7 days or less	Minimal effects clearing in less than 24 hours

TABLE II

Summary of Ocular Lesion Scores of Two Unicharge Propellants in the Primary Eye Irritation

PH 421-US-001, 002-91

Bis-(2,2-Dinitropropyl) Acetal with Diphenyl Amine Stabilizer

Rabbit No. Sex	Observations	Hours 1	24	48	72
5501 M	Cornea	0	0	0	0
	Iris	0	0	0	0
	Conjunctivae	2,0	0,0	0,0	0,0
5502 M	Cornea	0	0	0	0
	Iris	0	0	0	0
	Conjunctivae	2,0	0,0	0,0	0,0
5503 M	Cornea	0	0	0	0
	Iris	0	0	0	0
	Conjunctivae	2,0	0,0	0,0	0,0
5504 F	Cornea	0	0	0	0
	Iris	0	0	0	0
	Conjunctivae	2,0	0,0	0,0	0,0
5505 F	Cornea	0	0	0	0
	Iris	0	0	0	0
	Conjunctivae	1,0	0,0	0,0	0,0
5506 F	Cornea	0	0	0	0
	Iris	0	0	0	0
	Conjunctivae	1,0	0,0	0,0	0,0

Cornea = degree of opacity
Iris = degree of iritis
Conjunctivae = redness, chemosis

TABLE II (continued)

Summary of Ocular Lesion Scores of Two Unicharge Propellants in the Primary Eye Irritation

PH 421-US-001, 002-91

Bis-(2,2-Dinitropropyl) Formal without Diphenyl Amine Stablizer

Rabbit No. Sex	Observations	Hours 1	24	48	72
5291 M	Cornea	0	0	0	0
	Iris	0	0	0	0
	Conjunctivae	2,0	0,0	0,0	0,0
5292 M	Cornea	0	0	0	0
	Iris	0	0	0	0
	Conjunctivae	1,0	0,0	0,0	0,0
5293 M	Cornea	0	0	0	0
	Iris	0	0	0	0
	Conjunctivae	2,0	0,0	0,0	0,0
5294 F	Cornea	0	0	0	0
	Iris	0	0	0	0
	Conjunctivae	1,0	0,0	0,0	0,0
5295 F	Cornea	0	0	0	0
	Iris	0	0	0	0
	Conjunctivae	1,0	0,0	0,0	0,0
5296 F	Cornea	0	0	0	0
	Iris	0	0	0	0
	Conjunctivae	1,0	0,0	0,0	0,0

Cornea = degree of opacity
Iris = degree of iritis
Conjunctivae = redness, chemosis

TABLE III

Summary of Positive Scores of Two Unicharge Propellants in the Primary Eye Irritation

PH 421-US-001, 002-91

Bis-(2,2-Dinitropropyl) Acetal with Diphenyl Amine Stabilizer

	Hours			
	1	24	48	72
Cornea				
Opacity	0/6	0/6	0/6	0/6
Iritis	0/6	0/6	0/6	0/6
Conjuntivae				
Redness	4/6	0/6	0/6	0/6
Chemosis	0/6	0/6	0/6	0/6

Bis-(2,2-Dinitropropyl) Formal without Diphenyl Amine Stablizer

	Hours			
	1	24	48	72
Cornea				
Opacity	0/6	0/6	0/6	0/6
Iritis	0/6	0/6	0/6	0/6
Conjuntivae				
Redness	2/6	0/6	0/6	0/6
Chemosis	0/6	0/6	0/6	0/6

Table IV. Summary of Body Weights (g) of Two Unicharge Propellants in the Primary Eye Irritation

PH 421-US-001, 002-91

Bis-(2,2-Dinitropropyl) Acetal with Diphenyl Amine Stabilizer

Animal Number	Sex	Initial	Final
5501	M	1741	1788
5502	M	1821	1881
5503	M	1934	1990
5504	F	1914	1957
5505	F	1885	1927
5506	F	1992	2045

Bis-(2,2-Dinitropropyl) Formal without Diphenyl Amine Stablizer

5291	M	1841	1973
5292	M	1758	1798
5293	M	1815	1927
5294	F	1760	1838
5295	F	1926	1995
5296	F	1777	1826

QUALITY ASSURANCE UNIT STATEMENT

Study Nos.: PH 421-US-001-91
PH 421-US-002-91

Study Director: Victor T. Mallory

The Quality Assurance Unit conducted the inspections listed below and reported the results to the study director and to management on the dates indicated.

The following inspections were performed:

Interval	Date
In Life Phase	November 22, 1991 November 22, 1991
Reporting Phase	January 29, 1992

Date QAU Report Issued

To Study Director	To Management
January 29, 1992	January 29, 1992

Quality Assurance

Jan 29, 1992
Date

COMPLIANCE STATEMENT

This study was conducted in compliance with the Principles of Good Laboratory Practices (GLP) as promulgated by the following regulatory agencies.

EPA as stated in the Federal Register, 40 CFR Parts 160 and 792.

Organization for Economic Co-operation and Development Guidelines for Testing Chemicals (OECD), ISBN 92-64-12221-4, adopted by the council at its 535th meeting on May 12, 1981.

U.S. Food and Drug Administration as stated in 58 CFR Part 21.

Study Nos.: PH 421-US-001-91
PH 421-US-002-91

To the best of my knowledge, this study was conducted in accordance with applicable Good Laboratory Practice regulations; there were no deviations from these regulations that impacted on study conclusions.

Study Director

Date May 29, 1992